

Insect chemical ecology research in the United States Department of Agriculture–Agricultural Research Service^{†‡§}

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Abstract: This multi-author paper reviews current work by USDA-ARS scientists in the field of chemical ecology. Work with pheromones, the discovery and development of the codling moth kairomone, studies on insect–plant interactions and chemically mediated tritrophic plant–insect interactions have led to practical methods for control of important insect pests.

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1 INTRODUCTION

Discovery and development of environmentally safe chemicals to manage the behavior of insect pests and their natural enemies is the primary mission of insect chemical ecologists in the Agricultural Research Service (ARS). Here, ARS investigators from four different regions of the country summarize a variety of approaches to applied chemical ecology. Research on insect pheromones has long been an important focus of this effort,¹ and pheromones continue to be the cornerstone of insect behavioral manipulation, as exemplified in Section 2. The extent to which plants are signal sources guiding insect pests to find and accept a potential host has become increasingly apparent in recent years. Section 3 describes the discovery of a particular phytochemical that is exploited by codling moth females and males to find host-plants, and how the synthetic kairomone is becoming part of the arsenal for codling moth management. In the first part of Section 4, a series of studies are detailed showing that green leaf volatiles (GLVs) are common, if not ubiquitous, modulators of phytophagous insect behavior. Then, discovery of a GLV-based plant attractant and the male-produced

aggregation pheromone for the Colorado potato beetle (CPB) are described, semiochemical breakthroughs with the potential to transform CPB management. Finally in Section 5, the now substantial evidence is reviewed showing that insect attack induces plants to, in effect, call parasitoids and predators to their defense—a third trophic level of plant protection beyond that once imagined by scientists.

2 PHEROMONE CHEMISTRY OF COLEOPTERANS AND HYMENOPTERANS

Pheromones have become important tools for monitoring and controlling agricultural pest populations. This technology has been developed most for moths and certain beetle groups. However, basic pheromone knowledge is still lacking for many economically important insect species, both with respect to chemistry and with respect to mate-finding biology. An ongoing project at The National Center for Agricultural Utilization Research (NCAUR) has been to develop such knowledge and to explore the potential for using the new pheromones in practical insect management.

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Significant progress has been made over the last few years on a number of previously unstudied pheromone systems. A typical approach has involved gas chromatographic (GC) comparison of volatiles collected from males and females combined with electroantennographic detector (GC-EAD) analysis of these extracts. Sex-specific compounds that stimulate intense antennal responses are considered likely pheromone components. These compounds are identified by spectrometric methods (primarily MS and NMR) and are then synthesized. The synthetic compounds are subsequently used to verify behavioral activity, typically in the field. The increased sensitivity of modern analytical methods has made it possible to detect, evaluate antennal activity and identify key compounds even if only small numbers of individuals are available (in favorable cases, fewer than one hundred). Sufficient numbers of insects can often be obtained from field collections, thus obviating labor-intensive rearing programs. Recent results for five pheromone systems are summarized below.

2.1 Sap beetles

The sap beetle, *Coleopterus truncatus* Randall (Coleoptera: Nitidulidae), is a known vector of *Ceratocystis fagacearum* (Bretz) Hunt, the fungus that causes the oak wilt disease. The beetles are minute in size and highly cryptic in habits, and a pheromone for this species would be useful for monitoring its abundance and field activity patterns, particularly with respect to disease transmission.

Collections of volatiles from groups of males and females feeding on a wheat-germ/brewer's-yeast artificial diet revealed three male-specific compounds that produced strong responses from the antennae of both sexes. These were identified as methyl-branched, unsaturated hydrocarbons (1–3, Fig 1), and the synthesized compounds were attractive to both sexes in field tests, supporting their characterization as an aggregation pheromone.² Nitidulids of the genus *Carpophilus* also use alkyl-branched unsaturated hydrocarbons as aggregation pheromones.³ An important feature for both genera is that the pheromones

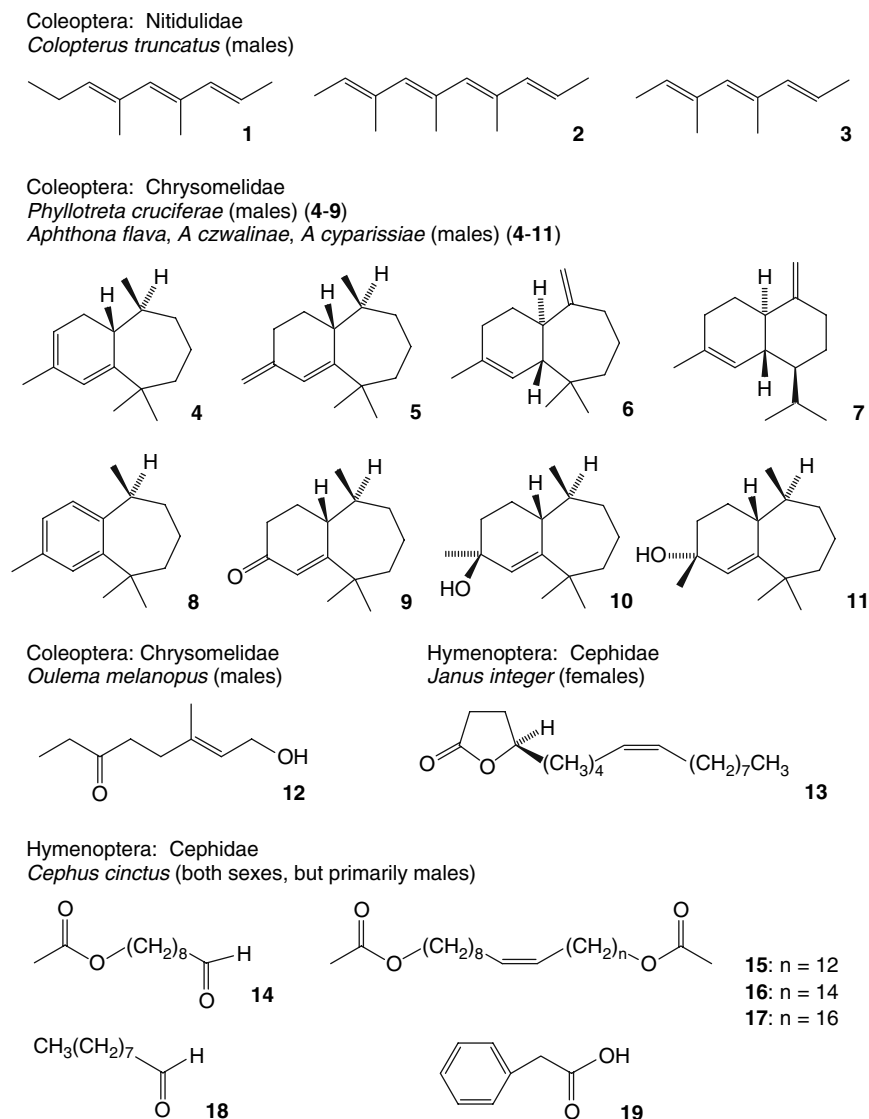


Figure 1. Structures of insect-derived compounds recently identified at The National Center for Agricultural Utilization Research (NCAUR). The producing sex is noted in parentheses.

act synergistically with the volatiles emitted by various host-related yeasts and fungi.

2.2 Flea beetles

Flea beetles (Coleoptera: Chrysomelidae, subfamily Alticinae) are a large group that includes both serious crop pests and beneficial species. Adults of most species feed on host plant foliage, while the larvae bore into the roots. No pheromone structures had been published for this group, but Peng *et al.*⁴ reported that males of the crucifer flea beetle, *Phyllotreta cruciferae* (Goeze), feeding on host foliage (*Brassica napus* L.) attracted both males and females in the field, indicating the existence of a male-produced aggregation pheromone. This species is a major pest of rapeseed and canola in the prairies of the northern USA and Canada. Chemical studies were begun at NCAUR with *P. cruciferae* and, subsequently, with three beneficial species of *Aphthona* [*A. flava* Guillebeau, *A. czwalinae* (Weise), and *A. cyparissiae* (Koch)], which had been introduced into the USA as biological control agents of leafy spurge (*Euphorbia esula* L.), a serious rangeland weed.

Comparisons of collections of volatiles from males and females of *P. cruciferae* feeding on canola or cabbage revealed six male-specific compounds, which were identified as the sesquiterpenoids 4–9 in Fig 1.⁵ Intriguingly, males of all three of the *Aphthona* species also emitted compounds 4–9, plus two other compounds (10 and 11, Fig 1) not detected from *P. cruciferae*. All except 7 and 8 are new natural products. Ratios of the compounds were quite consistent within species but differed between species, as might be expected for pheromones of closely related taxa. In preliminary GC-EAD tests, the compounds elicited antennal responses from both sexes, and this was consistent with the initial field results.⁴ Racemic forms of 4, 5 and 8–11 have been synthesized (Bartelt RJ, unpublished). A mixture containing synthetic racemic 4, 5, 8 and 9 and enantiomerically pure 7, which was isolated from citronella oil, was attractive to *P. cruciferae* in a 2001 field test near Saskatoon, Saskatchewan, Canada (Soroka JJ, pers comm).

2.3 Cereal leaf beetle

This chrysomelid species (*Oulema melanopa* L.) was introduced into the USA from Europe about 1960. It quickly became a serious pest of oats and other small grains. As with the flea beetles, volatiles were collected from males and females feeding on host plant (oat) foliage; however, male/female differences were not obvious from visual comparisons of gas chromatograms. Nevertheless, the highly sensitive GC-EAD technique did reveal a male-specific compound, present in very low amounts, that was readily detected by antennae of both sexes,⁶ again suggesting a male-produced aggregation pheromone. Knowing the GC characteristics of the compound allowed collection procedures to be improved (especially silanization of glass surfaces to reduce sample adsorption),

and enough material was accumulated for spectral analysis (NMR was essential). The compound was identified as a hydroxyketone (12, Fig 1) and was synthesized.⁶ Future research will include field testing of this compound.

2.4 Currant stem girdler sawfly

The currant stem girdler (*Janus integer* Norton, Hymenoptera: Cephidae) is an occasional pest of red currant (*Ribes* spp) in North America and is of economic importance in the state of Washington. Pheromones in the Cephidae were previously unexplored. Collections of volatiles from males and females were analyzed by GC-EAD. A female-specific compound was detected that caused an intense response in male (but not female) antennae, a pattern indicative of a sex pheromone. The compound was identified as a lactone (13, Fig 1), and the racemic form was synthesized.⁷ This synthetic compound was found to be attractive to males in the initial field test (James DG, pers comm). The natural compound was subsequently determined to be the (*R*) enantiomer (shown in Fig 1) by chiral GC and chiral synthesis (Petroski RJ, pers comm).

2.5 Wheat stem sawfly

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is a serious pest of winter wheat (*Triticum aestivum* L.) in the northern Great Plains of the USA and Canada. GC-EAD analysis of collections of volatiles from males and females revealed a situation very different from that with the cephid, *J. integer* (Section 2.4). Compounds eliciting antennal responses were again found, but the same ones were emitted from both sexes (in somewhat different ratios and in greater overall amounts from males), and they stimulated the antennae of both sexes. The compounds included an unusual aldehyde acetate (14, Fig 1), simple aldehydes such as nonanal (18) and phenylacetic acid (19). Compound 14 was synthesized and was found to be attractive to sawflies of both sexes in the initial field test.⁸

Compound 14 is apparently formed by cleavage of the double bond in three unusual cuticular lipids (alkenediol diacetates 15–17, Fig 1) upon exposure to air. These diesters were isolated from both sexes, but they are about 10 times more abundant in males. The structures were proven by synthesis.⁹ Further research will be required to fully understand this pheromone system, but its unusual nature is already obvious.

3 DISCOVERY AND DEVELOPMENT OF THE CODLING MOTH KAIROMONE

Semiochemicals have played a major role in the management of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in pome fruits and walnuts for over 30 years. The sex pheromone of *C. pomonella* has been widely used in lures to monitor population density and phenology and in a variety of formulations

and dispenser types to achieve mating disruption.¹⁰ However, the use of the insect's sex pheromone is limited because it is only attractive and/or disruptive to males. Conversely, larvae and both sexes of adults are attracted to the odor of apples^{11,12} and specifically to one of its volatile components, (*E,E*)- α -farnesene.^{13,14} Unfortunately, a key element limiting the development of (*E,E*)- α -farnesene as a semiochemical tool for *C pomonella* management has been its instability and rapid chemical breakdown.¹⁵ Our objectives were to identify other host-plant volatiles attractive to *C pomonella* and to evaluate their potential utility in orchard pest management.

Ninety-two host-plant volatiles were identified in pome fruits by GC-MS and chosen for behavioral screening.¹⁶ Twenty-three blends, composed of two to nine pome fruit volatiles sharing a common carbon-chain length (from four to 15 carbons) and/or alcohol, aldehyde or ester moiety, were formulated. Of the 23 blends tested, only the 'C-10 ester blend' (a blend of methyl and ethyl esters of 10-carbon acids) attracted *C pomonella* to traps. The principal attractive constituent in this synthetic blend was found to be ethyl (*E,Z*)-2,4-decadienoate (Et-*E,Z*-DD).¹⁶ The kairomonal activity of Et-*E,Z*-DD to *C pomonella* neonates was established in a series of laboratory 'choice' and 'non-choice' bioassays.¹⁷ Et-*E,Z*-DD was attractive at levels 1/1000th of the concentrations effective for (*E,E*)- α -farnesene in dual-choice Y-tube bioassays.

Alkyl (*E,Z*)-2,4-decadienoates are known to be important volatiles of ripening and fully ripe pears, and are responsible for the characteristic aroma of 'Bartlett' pear.¹⁸ In contrast, Et-*E,Z*-DD has not been found in the odor of apple fruits, although a broad range of volatiles, primarily other esters and sesquiterpenes, have been identified from apple varieties as

they mature through the season.^{19–21} In addition, alkyl (*E,Z*)-2,4-decadienoates have not been isolated from the odor of immature pome fruit (Buttery RG, pers comm) or from pear leaves.^{22,23} Alkyl (*E,Z*)-2,4-decadienoates are however, released from immature pear fruit infested by codling moth (Buttery RG, pers comm).

In field dose-response tests with adults, Et-*E,Z*-DD exhibited a response threshold and attractiveness for *C pomonella* comparable with its synthetic sex pheromone, codlemone, over a range of doses. No endemic moth species other than *C pomonella* or 'non-target' beneficial insects were caught in Et-*E,Z*-DD-baited traps. However, low numbers of stink bugs (Pentatomidae) and yellow jacket wasps (Vespididae) were captured at high doses on rubber septa substrates, consistent with coincidental reports of attractiveness of Et-*E,Z*-DD to these insects.^{24,25} GC-EAD analyses of entrained headspace volatiles of ripe Bartlett pear odor revealed that both male and female *C pomonella* antennae responded specifically to the Et-*E,Z*-DD peak.¹⁶ No other obvious and temporally correlated EAD-depolarization responses were observed to the other 15 FID-detected peaks.

Kairomone-baited traps detected, and tracked the seasonal *C pomonella* flights accurately in both walnut and apple orchards, but were only moderately effective in pear orchards.¹⁶ Et-*E,Z*-DD lures attracted males, and virgin and mated females. Lures loaded with 1 mg of Et-*E,Z*-DD remained attractive for several months. Et-*E,Z*-DD was generally equivalent to pheromone in attraction and capture efficacy of *C pomonella* over the entire season in walnut but only during the first flight in conventionally-managed apple orchards (Fig 2). Et-*E,Z*-DD was a much more effective attractant than pheromone in monitoring *C pomonella* in orchards treated with pheromone for mating disruption, both in

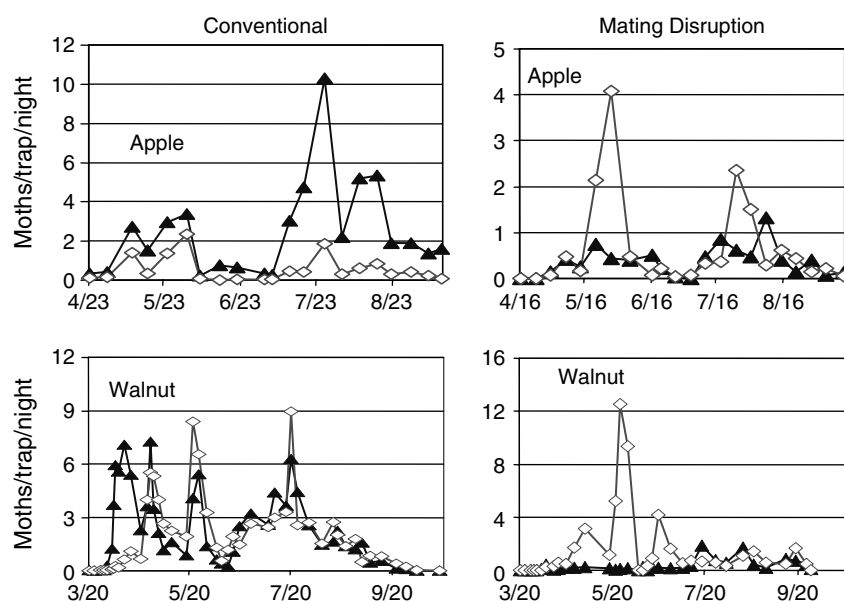


Figure 2. Comparison of the capability of (◇) kairomonal ethyl (*E,Z*)-2,4-decadienoate lure and (▲) pheromonal codlemone lure to monitor the emergence and flight activity of codling moth populations endemic to Washington State apple and Californian walnut orchards under conventional and mating disruption management in the 2000 season.

walnut and in apple orchards, throughout the season (Fig 2).

Typical flight patterns were observed in monitoring each generation of *C pomonella* in apple and walnut (Fig 2). The kairomone lure tended to catch the first moths of the season somewhat later than codlemone lures. At the beginning of each moth flight, the kairomone lure caught a higher proportion of males than females and a lower proportion of mated than virgin females. During the peak flight of each generation, the kairomone lure caught equal numbers of each sex and greater than 80% of the females were mated. Toward the end of each flight, the kairomone lure caught more females than males and the proportion of mated females remained high. This pattern was similar in both conventional and mating-disruption orchards. However, the percentage of mated females was somewhat lower in mating disruption than in conventional orchards but still averaged greater than 60%. Improved predictions of *C pomonella* egg hatch based from the date of capture of mated females and assessing local density from the number of female moths captured using the kairomone lure has been successfully demonstrated over several seasons, and will likely be adopted by growers.

Direct management of *C pomonella* with kairomone-based products may also be possible, especially in walnuts where the kairomone is more attractive and the damage threshold is higher than in pome fruit. Formulations of Et-*E*,*Z*-DD mixed with insecticides in 'attract and kill' paste drops (attracticide) and bait stations are being tested for male and female annihilation. Management of *C pomonella* by mass trapping of both sexes looks promising. Liquid formulations to control larvae using reduced rates of insecticides are also being developed. A kairomone–insecticide cocktail applied to an orchard canopy could increase the time neonates spend walking on foliage prior to entry into fruit or nuts. Extending the period of larval wandering could increase natural mortality and exposure to biological control agents or to the residues of standard or microbial insecticides.

4 INSECT–PLANT INTERACTIONS

As seen in Sections 2 and 3, volatile chemicals emitted by potential host plants and conspecifics play important roles in directing insects to suitable food sources and reproductive sites. Chemicals emitted by plants may attract or deter insects orienting to them. Insect-produced pheromones are detected against a background of odorous molecules released by host and non-host plants; thus, insects must have specialized receptors for individual components in order to detect signals critical to their survival and reproduction.²⁶

4.1 Interactions between plant emissions and pheromones

Insect pheromones are generally comprised of blends of chemicals emitted by signaling insects. While

pheromone blends may be active alone, the attractiveness of the blends may be enhanced by specific host-plant volatiles. A ubiquitous group of plant compounds, called green leaf volatiles (GLVs),²⁷ may modify behavioral responses of insects to their pheromones, depending on the insect species and the context in which they are encountered.^{28–32}

GLVs are six-carbon alcohols, aldehydes and their derivatives, eg acetates, which are produced by plants as a result of oxidative degradation of surface lipids.²⁷ The effects of GLVs on insect pheromone responses were first observed in the cotton boll weevil, *Anthonomus grandis* Boh (Coleoptera: Curculionidae).²⁸ In competitive field tests, release of the GLVs (*E*)-2-hexen-1-ol with the boll weevil aggregation pheromone (grandlure) resulted in more

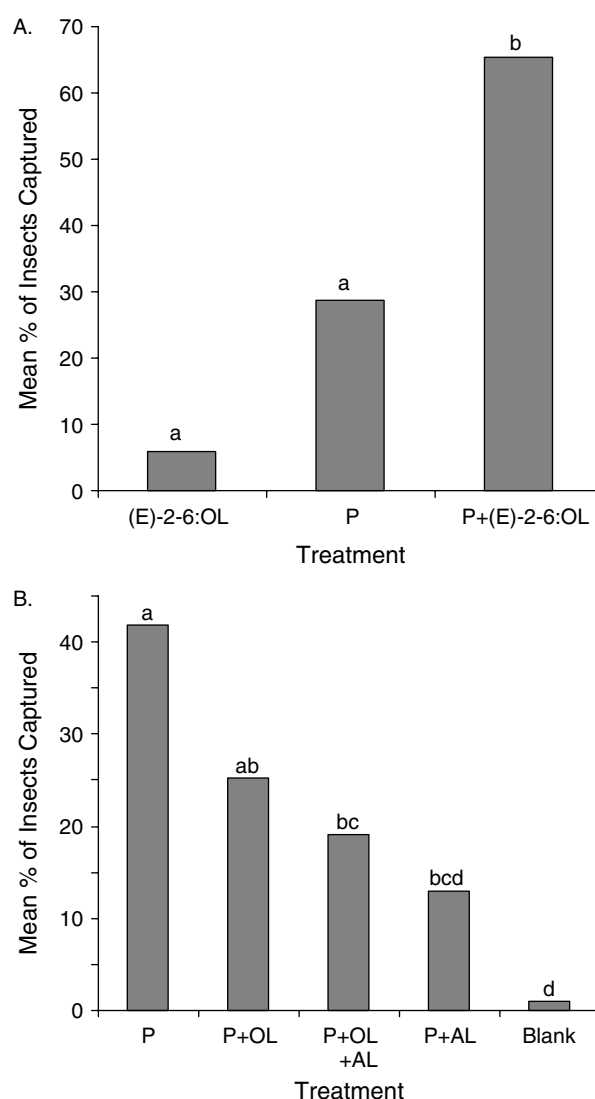


Figure 3. Contrasting effects of green leaf volatiles (GLVs) on pheromone responses of insects associated with: (A) broad-leaved plants, e.g. the cotton boll weevil *Anthonomus grandis*. (Coleoptera: Curculionidae) (P = pheromone (grandlure); (E)-2-6:OL = (*E*)-2-hexen-1-ol)²⁸ and, (B) insects associated with the boles of pine trees, an environment rich in monoterpenes, e.g. the bark beetle *Ips avulsus* (P = *Ips* pheromone; OL = 1-hexanol; AL = hexanal).³¹ Bars with different letters are significantly different ($P < 0.05$, Duncan's multiple range test).

than twice as many weevils captured in traps compared to grandlure alone, although (*E*)-2-hexen-1-ol alone was relatively inactive (Fig 3A). Other GLVs (ie (*Z*)-3-hexen-1-ol and 1-hexanol) also enhanced responses to grandlure, while responses to grandlure were not enhanced by (*E*)-2-hexenal or the GLVs analog, (*Z*)-2-hexen-1-ol. Single-cell recordings from antennal olfactory receptors in the boll weevil revealed neurons that respond selectively to GLVs.²⁸ Pheromones of other insects associated with broad-leafed deciduous plants were also enhanced by GLVs.^{29,32,33} But what about insects that feed and release their pheromones from the boles of pine trees, an environment rich in monoterpenes, distant from broad-leafed plants?

Bark beetles infesting pines release aggregation pheromones from their frass. Responses to these pheromones are often enhanced by host-tree monoterpenes or modified by pheromone emissions of cohabiting species. The discovery that non-host volatiles interrupted responses of bark beetles to their pheromones was first made by Dickens *et al*^{30,31} who demonstrated interruption of pheromone responses by GLVs in a guild of three bark beetle species cohabiting pines in the southern USA (Fig 3B). It was hypothesized that the effects of GLVs might not only aid in the orientation of bark beetles to pheromone-producing conspecifics on pine trees, but also provide a chemical signal for avoidance of non-hosts and predators associated with them. Since this initial discovery, the general nature of disruption of pheromone responses of bark beetles by GLVs and other non-host volatiles has been verified by other researchers in Canada,^{34,35} Sweden³⁶ and the USA.³⁷ Thus, GLVs may be useful in the protection of trees from bark beetles around the world.

4.2 Host-plant attraction in Colorado potato beetle

Host finding by the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), a serious pest of solanaceous crops, has been the subject of numerous investigations since McIndoo³⁸ first showed the attractiveness of potato foliage to the beetle in an olfactometer. Only recently have synthetic blends of chemicals released by potatoes that attract *L. decemlineata*³⁹ and a male-produced aggregation pheromone been identified.^{40,41} Both discoveries were facilitated by novel approaches that may be applicable to discovery of other insect attractants.

Chemical emissions by intact, mechanically damaged, or insect-damaged potato plants had been reported.^{42,43} Based on these investigations, a blend comprised of equal amounts of many of these previously identified chemicals was formulated.³⁹ Using serial dilutions of this blend and GC-EAD preparations of adult *L. decemlineata*, selective sensitivity of the insect to nine compounds contained in the blend was demonstrated. Because generalist [*Podisus maculiventris* (Say)] and specialist [*Perillus bioculatus* (F)] predators (Hemiptera, Heteroptera: Pentatomidae)

were known for CPB, antennal responses of both predators were used to provide additional clues as to chemicals used by their prey. Indeed, seven of the nine compounds detected by antennal receptors of *L. decemlineata* were also detected by the predators.³⁹

Initial laboratory behavioral bioassays demonstrated attraction of the generalist predator and adult *L. decemlineata* to a blend of five compounds: (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol, nonanal, (±)-linalool and methyl salicylate.³⁹ Subsequent behavioral studies showed adult *L. decemlineata* were attracted to several two- and three-component blends comprised of (*Z*)-3-hexenyl acetate (**20**), (±)-linalool (**21**) and methyl salicylate (**22**) (Fig 4A).⁴⁴ A blend of compounds **20–22** was also attractive to *L. decemlineata* larvae.⁴⁵

Discovery of a male-produced aggregation pheromone for *L. decemlineata*^{40,41} broke a long-time

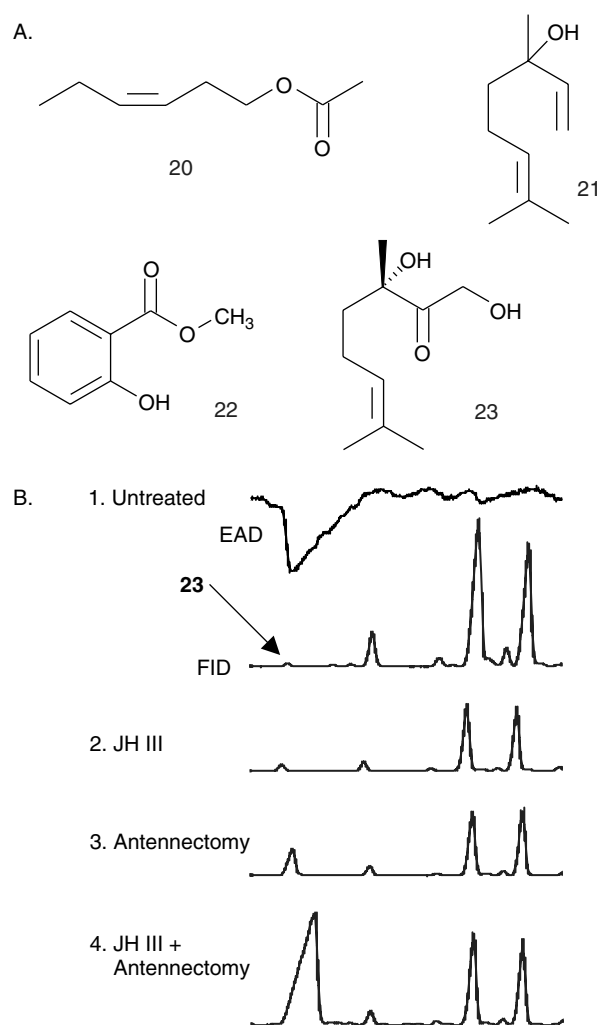


Figure 4. Semiochemistry of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae): (A) attractive plant volatiles (**20–22**; (*Z*)-3-hexenyl acetate, (±)-linalool and methyl salicylate, respectively) and the male-produced aggregation pheromone (**23**; (*S*)-3,7-dimethyl-2-oxo-oct-6-ene-1,3-diol) and, (B) production of **23** by (1) untreated *L. decemlineata* males, (2) males topically treated with juvenile hormone III (JH III), (3) antennectomized males, and (4) antennectomized males also treated with JH III. (Electroantennogram detection (EAD) is shown for volatiles from untreated males only; flame ionization detector (FID) responses are shown for all treatments)⁴⁰.

paradigm for a female-produced sex attractant for this insect.^{46,47} In fact, the very existence of a volatile sex attractant for it had been disputed.⁴⁸ Using coupled GC-EAD preparations, responses of antennal receptors of *L. decemlineata* were recorded to volatiles collected from intact potato plants, mechanically damaged plants and plants on which males or females were feeding.⁴⁰ Several EAD responses were coincident with FID peaks to certain plant volatiles for all collections. One EAD response was present only for volatiles collected from males feeding on the plant; however, a coincident FID peak was never observed. While subsequent collections of volatiles produced by larger numbers of males feeding on potato foliage revealed a small, barely distinguishable peak for the male-specific volatile (Fig 4B-1), the amounts collected were insufficient for isolation and identification.

The problem of insufficient quantities of the male-specific compound for characterization was solved by application of physiological techniques previously described for the boll weevil, *A. grandis*.⁴⁹ There we showed that topical application of a juvenile hormone analog (JHA), antennectomy, and the combination of JHA application and antennectomy increased quantities of pheromone released by male weevils. Application of a similar strategy to production of the *L. decemlineata* male-specific component led to increased quantities of the compound of up to 200-fold for the combined treatment of JH application and antennectomy⁴⁰ (Fig 4B-4). This finally enabled collection of quantities of the male-specific compound for its chemical identification as (S)-3,7-dimethyl-2-oxo-oct-6-ene-1,3-diol (**23**, Fig 4A),^{40,41} a structure unique for an insect pheromone. As for the boll weevil,⁴⁹ these results again imply that pheromone release is controlled by antennal sensory input via a negative feedback loop. Laboratory bioassays showed **23** to be an aggregation pheromone, attracting both males and females.⁴⁰

The plant attractant blend has been tested as a component of an attracticide⁵⁰ and in the 'push-pull' strategy of insect control (Martel JW, Alford AR and Dickens JC, unpublished). Our initial studies demonstrated the usefulness of the plant attractant in *L. decemlineata* management and these approaches should lead to decreased levels of pesticide needed for regulation of pestiferous populations of this insect.

5 CHEMICALLY MEDIATED TRITROPHIC PLANT-INSECT INTERACTIONS

Biological control of insect pests has become increasingly important in agriculture because of the need to minimize the amount of toxic chemicals released in the environment. To develop effective and reliable methods for biological control with natural enemies, understanding the chemically mediated interactions of parasitoids or predators with their hosts or prey, as well as the plants on which the hosts feed, is essential.

The single most important activity for a parasitoid female is to find suitable hosts to ensure the survival of her progeny. Many parasitoids attack more than one host species, and even specialists must often locate their hosts on more than one plant species. Thus, parasitoid females must be highly versatile to detect and use the various cues that indicate the location of their hosts. Several studies have shown that learning plays an important role in parasitoid host foraging behaviors.^{51–60} Lewis and Tumlinson⁶¹ discovered that females of *Microplitis croceipes* (Cresson), a parasitoid of *Heliothis* and *Helicoverpa* larvae, associatively learn to recognize odors by linking them to the presence of non-volatile chemicals in host feces. This ability to learn a variety of chemical cues associated with the presence of their host is a great advantage in locating their hosts in diverse habitats.

Feeding by beet armyworm, *Spodoptera exigua* (Hübner), larvae on corn seedlings results in the release of large amounts of volatile organic compounds by the plants. Females of the generalist parasitoid *Cotesia marginiventris* (Cresson) learn to take advantage of these volatiles to locate hosts after experiencing them in association with hosts or host by-products.⁶² Several studies have shown that plants respond to insect feeding damage by releasing volatile organic compounds that allow natural enemies of the herbivores to distinguish between infested and non-infested plants and thus aid in location of prey or hosts. The biosynthesis and/or release of these volatiles are elicited by interaction of compounds in the saliva or oral secretion of the herbivores with damaged plant tissues. Such chemically mediated tritrophic plant-insect interactions have been documented for several crop plants, including lima beans, cucumbers, corn and cotton.^{62–66}

An undamaged plant maintains a baseline level of volatile metabolites that are released from the surface of the leaf and/or from accumulated storage sites in the leaf. These constitutive chemical reserves often accumulate to high levels in specialized glands or trichomes.⁶⁷ They include monoterpenes, sesquiterpenes and aromatics. With damage, the profile of volatiles emitted from the foliage markedly changes. In cotton, breakage of leaf glands causes stored terpenes to be released in much higher levels, and the emission of lipoxygenase pathway green leaf volatiles also increases. The release of these metabolites correlates closely with leaf damage from insect feeding.⁶⁸ This is in contrast to a subset of terpenes, indole and hexenyl acetate that are also released in much higher levels with insect feeding, but in a diurnal cycle that is decoupled from short-term insect damage, with low emissions at night and high levels during the periods of maximal photosynthesis. Chemical labeling studies with [¹³C]carbon dioxide have established that these induced compounds are synthesized *de novo*, specifically in response to insect damage, and are not stored in the plant.^{67,69} Similar compounds are emitted in response to insect herbivore

damage in several agricultural species that have been studied, including cucumber, apple, lima bean, corn and cotton.⁷⁰

In addition to the release of volatiles at the site of herbivore feeding, analysis of volatile emissions from unharmed leaves of insect-damaged plants has established that both corn and cotton also respond systemically.^{71,72} The chemical blend of volatiles from undamaged cotton leaves differs from the volatiles collected from the entire plant.⁷² The terpenoids that are synthesized *de novo* in cotton leaves in response to herbivore damage are also released systemically from undamaged leaves of an herbivore-injured plant.^{67,69} The terpenoids that did not incorporate ¹³C when damaged cotton plants were exposed to [¹³C]carbon dioxide were not released systemically. Labeling studies with [¹³C]carbon dioxide and analysis of the systemically released volatiles by gas chromatography–mass spectroscopy (GC-MS) showed that a high level of ¹³C was rapidly incorporated into these compounds, indicating that they are synthesized *de novo* at the site of release.⁷³ Thus a signal, transmitted from the site of damage to distal undamaged leaves to trigger synthesis and release of volatile compounds, serves as a mechanism for amplifying the message that the plant is under herbivore attack.

The chemical signals released by plants also may be herbivore specific. Females of the parasitoid *Cardiochiles nigricipes* Viereck clearly distinguished between tobacco plants damaged by their host, *Heliothis virescens* (F), and those infested by a non-host, *Helicoverpa zea* (Boddie).⁶⁶ This preference of the parasitoids for plants damaged by their hosts persisted even when all caterpillars and damaged leaves were removed from the plants, leaving only the undamaged leaves from which volatiles are released systemically. Furthermore, GC-MS analysis of volatiles released by plants damaged by the two herbivore species clearly showed differences in the proportions of compounds in blends induced by *H virescens* and those induced by *H zea* feeding on both cotton and tobacco. This suggests that different herbivore species produce different elicitors. However, analysis of the oral secretions of *H virescens* and *H zea* revealed the presence of the same fatty acids and fatty acid-amino acid conjugates previously identified in the oral secretions of *S exigua* larvae.^{74,75} Even more interesting is the observation that volatile blends emitted by tobacco plants in response to feeding by *H virescens* larvae differ significantly between night and day and that mated *H virescens* females were repelled and deterred from ovipositing by the nocturnal volatiles.⁷⁶

Elicitors of plant volatiles have been identified in the oral secretions of several species of insect herbivores. Mattiacci *et al* reported that a β -glucosidase in the saliva of *Pieris brassicae* L. caterpillars elicits the release of volatiles from cabbage leaves.⁷⁷ Alborn

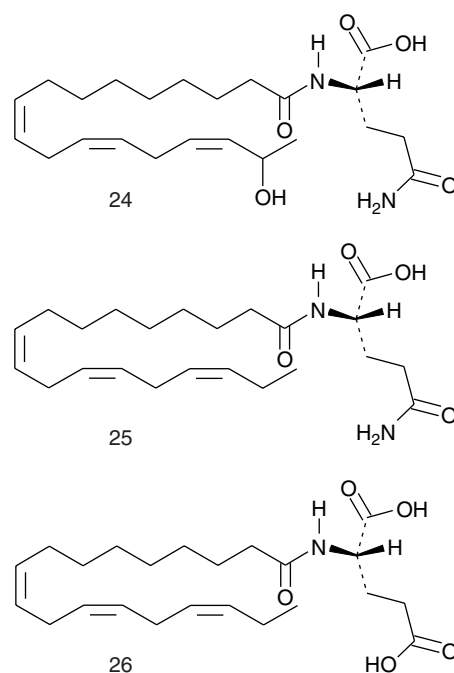


Figure 5. Volicitin (*N*-[17-hydroxylinolenoyl]-L-glutamine) (**24**), a compound in the saliva of *Spodoptera exigua* larvae that elicits blends of volatile terpenoids and indole from damaged corn (*Zea mays*), and other elicitors (**25** and **26**) found in lepidopteran larvae.

*et al*⁷⁸ identified volicitin (*N*-[17-hydroxylinolenoyl]-L-glutamine; **24**, Fig 5) as the major active elicitor in the oral secretion of beet armyworm, *S exigua*, larvae. Synthesized and natural **24** induce corn (*Zea mays* L) seedlings to release the same blend of volatile terpenoids and indole as is released when they are damaged by caterpillar feeding.⁷⁸ More recently Alborn *et al*⁷⁵ reported that the oral secretion of beet armyworm larvae contains several compounds analogous to **24**, including *N*-linolenoyl-L-glutamine (**25**, Fig 5), 17-hydroxylinolenic acid, linolenic acid and the linoleic acid analogs of all these compounds. These compounds have also been reported in the oral secretions of several other lepidopterous larvae.^{79,80} In addition, the oral secretion of tobacco hornworm, *Manduca sexta* (L), larvae contains **25** and *N*-linolenoyl-L-glutamate (**26**, Fig 5), but none of the 17-hydroxylinolenoyl analogs (Reference 81, and Alborn HA, Brennan M and Tumlinson JH, unpublished).

We have recently demonstrated that beet armyworm caterpillars synthesize **24** by adding a hydroxyl group and glutamine to linolenic acid obtained directly from the plant on which the caterpillar feeds.⁸² This strongly suggests that these molecules play an important, but still unknown, role either in the metabolism or some other process critical to the life of the herbivorous insects. It is also interesting to note that the plant is providing the linolenic acid, which is essential for most lepidopteran larvae,^{83–85} with which the insect makes an elicitor of plant chemical defenses, seemingly detrimental to the insect. The full implications of this are not yet understood.

6 SUMMARY

Chemical communication in insects does not occur in an environmental vacuum. Insect pheromones often, if not usually, interact synergistically with volatiles from host-plants or, less frequently, from other animals. Gas chromatography coupled with electroantennographic detection has been instrumental in pinpointing biologically meaningful signals among chemical background noise, yet behavioral bioassays remain essential to clarify the types of behavior elicited. For example, while green leaf volatiles are powerful synergists for attractant pheromones of insects feeding on broad-leaf plants, the same compounds are antagonistic to insect pests of conifers; however, GLVs elicit strong EAD responses in both groups of insects. Even with the importance of GLVs as modulators of insect behavior well established, who could have imagined the extent to which constitutive and induced plant volatiles guide natural enemies to potential hosts? Identification of the first elicitor of plant volatiles, volicitin, also revealed an intriguing coincidence in the evolution of the messengers themselves; not only is linolenic acid the precursor of GLVs, this plant-derived amino acid is also incorporated into the volicitin molecule. Still, an element of mystery remains in understanding herbivore-induced plant defense: Why should an insect produce a signal detrimental to itself? Recent evidence sheds some more light on this interaction; jasmonate and salicylate—plant-produced signals that activate plant defense genes after herbivory—simultaneously induce genes in insects that are associated with detoxification of plant allelochemicals.⁸⁶ At this juncture it is obvious that chemical ecology has matured tremendously since identification of the first insect pheromone in 1959,⁸⁷ and practical applications for insect semiochemicals are continually being devised. Yet, again, who could have imagined that an emerging role for this avenue of research would be the use of plants and insects as biosensors to detect threats, both natural and contrived? Finally, while gaps in our knowledge of insect chemical ecology are steadily being filled, the discipline remains fertile for discovery due to the overwhelming number of insect species and the webs of interactions between these creatures and their co-inhabitants of the planet.

REFERENCES

- Bierl BA, Beroza M and Collier CW, Potent sex attractant of the gypsy moth, *Porthetria dispar* (L): its isolation, identification, and synthesis. *Science (Washington)* **170**:87–89 (1970).
- Cossé AA and Bartelt RJ, Male-produced aggregation pheromone of *Colopterus truncatus*: structure, electrophysiological, and behavioral activity. *J Chem Ecol* **26**:1735–1748 (2000).
- Bartelt RJ, in *Pheromones of non-lepidopteran insects associated with agricultural plants*, ed by Hardie J and Minks AK, CABI Publishing, Wallingford, UK, pp 69–90 (1999).
- Peng C, Bartelt RJ and Weiss MJ, Male crucifer flea beetles produce an aggregation pheromone. *Physiol Entomol* **24**:98–99 (1999).
- Bartelt RJ, Cossé AA, Zilkowski BW, Weisleder D and Momany FA, Male-specific sesquiterpenes from *Phyllotreta* and *Aphthona* flea beetles. *J Chem Ecol* **27**:2397–2423 (2001).
- Cossé AA, Bartelt RJ and Zilkowski BW, Identification and electrophysiological activity of a novel compound emitted by male cereal leaf beetle. *J Nat Prod* **65**:1156–1160 (2002).
- Cossé AA, Bartelt RJ, James DG and Petroski RJ, Identification of a female-specific, antennally active volatile compound of the currant stem girdler. *J Chem Ecol* **27**:1841–1853 (2001).
- Cossé AA, Bartelt RJ, Weaver DK and Zilkowski BW, Pheromone components of the wheat stem sawfly: identification, electrophysiology, and field bioassay. *J Chem Ecol* **28**:391–407 (2002).
- Bartelt RJ, Cossé AA, Petroski RJ and Weaver DK, Cuticular hydrocarbons and novel alkenediol diacetates from wheat stem sawfly (*Cephus cinctus*): natural oxidation to pheromone components. *J Chem Ecol* **28**:385–405 (2002).
- Vickers RA and Rothschild GHL, 'Use of sex pheromones for control of codling moth'. In *World crop pests, tortricid pests: their biology, natural enemies and control*, ed by Vander Geest LPS and Evenhuis HH, Elsevier, Amsterdam, Netherlands, pp 339–370 (1991).
- Wearing CH, Connor PJ and Ambler KD, Olfactory stimulation of oviposition and flight activity of the codling moth *Laspeyresia pomonella*, using apples in an automated olfactometer. *N Z J Sci* **16**:697–710 (1973).
- Yan F, Bengtsson M and Witzgall P, Behavioral response of female codling moths, *Cydia pomonella*, to apple volatiles. *J Chem Ecol* **25**:1343–1351 (1999).
- Sutherland ORW, Hutchins RFN and Wearing CH, 'The role of the hydrocarbon α -farnesene in the behaviour of codling moth larvae and adults'. In *Experimental analysis of insect behavior*, ed by Barton Browne L, Springer, Berlin, Germany, pp 249–263 (1974).
- Hern A and Dorn S, Sexual dimorphism in the olfactory orientation of adult *Cydia pomonella* in response to an α -farnesene. *Entomol Exp Appl* **92**:63–72 (1999).
- Cavill GWK and Coggiola IM, Photosensitized oxygenation of α -farnesene. *Aust J Chem* **24**:135–142 (1971).
- Light DM, Knight AL, Henrick CA, Rajapaska D, Lingren B, Dickens JC, Reynolds KM, Buttery RG, Merrill G, Roitman J and Campbell BC, A pear derived kairomone with pheromonal potency that attracts male and female codling moth, *Cydia pomonella* (L). *Naturwissenschaften* **88**:333–338 (2001).
- Knight AL and Light DM, Attractants from Bartlett pear for codling moth, *Cydia pomonella* (L), larvae. *Naturwissenschaften* **88**:339–342 (2001).
- Jennings WG, Creveling RK and Heinz DE, Volatile esters of Bartlett pear. IV. Esters of *trans*-2-*cis*:4-decadienoic acid. *J Food Sci* **29**:730–734 (1964).
- Kakiuchi N and Moriguchi T, Composition of volatile compounds of apple fruits in relation to cultivars. *J Japan Soc Hort Sci* **55**:280–289 (1986).
- Mattheis JP, Fellman JK, Chen PM and Patterson ME, Changes in headspace volatiles during physiological development of Bisbee Delicious apple fruit. *J Agric Food Chem* **39**:1902–1906 (1991).
- Carle SA, Averill AL, Rule GS, Reissig WH and Roelofs WL, Variation in host fruit volatiles attractive to apple maggot fly, *Rhagoletis pomonella*. *J Chem Ecol* **13**:795–805 (1987).
- Miller RL, Bills DD and Buttery RG, Volatile components from Bartlett and Bradford pear leaves. *J Agric Food Chem* **37**:1476–1479 (1989).
- Scutareanu P, Drukker B, Bruin J, Posthumus MA and Sabelis MW, Volatiles from *Psylla*-infested pear trees and their possible involvement in attraction of anthocorid predators. *J Chem Ecol* **23**:2241–2260 (1997).
- Aldrich JR, Hoffmann MP, Kochansky JP, Lusby WR, Eger JE and Payne JA, Identification and attractiveness of a major pheromone component for Nearctic *Euschistus* spp stink bugs (Heteroptera: Pentatomidae). *Environ Entomol* **20**:477–483 (1991).
- Day SE and Jeanne RL, Food volatiles as attractants for yellowjackets (Hymenoptera: Vespidae). *Environ Entomol* **30**:157–165 (2001).

- 26 Dickens JC, Specialized receptor neurons for pheromones and host plant odors in the boll weevil, *Anthonomus grandis* Boh. (Coleoptera: Curculionidae). *Chem Senses* 15:311–331 (1990).
- 27 Visser JH, Van Straten S and Maarse H, Isolation and identification of volatiles in the foliage of potato, *Solanum tuberosum*, a host plant of the Colorado potato beetle, *Leptinotarsa decemlineata*. *J Chem Ecol* 5:13–25 (1979).
- 28 Dickens JC, Green leaf volatiles enhance aggregation pheromone of boll weevil, *Anthonomus grandis*. *Entomol Exp Appl* 52:191–203 (1989).
- 29 Dickens JC, Jang EB, Light DM and Alford AR, Enhancement of insect pheromone responses by green leaf volatiles. *Naturwissenschaften* 77:29–31 (1990).
- 30 Dickens JC, Billings RF and Payne TL, Green leaf volatiles: A ubiquitous chemical signal modifies insect pheromone responses. In *Insect chemical ecology*, ed by Hrdy I, SPB Acad Publ, Prague, Czech Republic, pp 277–280 (1991).
- 31 Dickens JC, Billings RF and Payne TL, Green leaf volatiles interrupt aggregation pheromone response in bark beetles infesting southern pines. *Experientia* 48:523–524 (1992).
- 32 Light DM, Flath RA, Buttery RG, Zalom FG, Rice RE, Dickens JC and Jang EB, Host plant green leaf volatiles synergize the synthetic sex pheromones of corn earworm and codling moth. *Chemoecol* 4:145–152 (1993).
- 33 Dickens JC, Smith JW and Light DM, Green leaf volatiles enhance sex attractant pheromone of the tobacco budworm, *Heliothis virescens* (Lep: Noctuidae). *Chemoecol* 4:175–177 (1993).
- 34 De Groot P and MacDonald LM, Green leaf volatiles inhibit response of red pine cone beetle *Conophthorus resinosae* (Coleoptera: Scolytidae) to a sex pheromone. *Naturwissenschaften* 86:81–85 (1999).
- 35 Huber DPW and Borden JH, Angiosperm bark volatiles disrupt response of Douglas-fir beetle, *Dendroctonus pseudotsugae*, to attractant-baited traps. *J Chem Ecol* 27:217–233 (2001).
- 36 Zhang Q-H, Schlyter F and Anderson P, Green leaf volatiles interrupt pheromone response of spruce bark beetle, *Ips typographus*. *J Chem Ecol* 25:2847–2861 (1999).
- 37 Poland TM and Haack RA, Pine shoot beetle, *Tomicus piniperda* (Col, Scolytidae), responses to common green leaf volatiles. *J Appl Entomol* 124:63–69 (2000).
- 38 McIndoo NE, An insect olfactometer. *J Econ Entomol* 19:545–571 (1926).
- 39 Dickens JC, Predator-prey interactions: olfactory adaptations of generalist and specialist predators. *Agric Forest Entomol* 1:47–54 (1999).
- 40 Dickens JC, Oliver JE, Hollister B, Davis JC and Klun JA, Breaking a paradigm: male-produced aggregation pheromone for the Colorado potato beetle. *J Exp Biol* 205:1925–1933 (2002).
- 41 Oliver JE, Dickens JC and Glass TE, (S)-3,7-Dimethyl-2-oxo-6-octene-1,3-diol: an aggregation pheromone of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). *Tetrahedron Lett* 43:2641–2643 (2002).
- 42 Bolter CJ, Dicke M, Van Loon JJA, Visser JH and Posthumus MA, Attraction of Colorado potato beetle to herbivore-damaged plants during herbivory and after its termination. *J Chem Ecol* 23:1003–1023 (1997).
- 43 Schütz S, Weißbecker B, Klein A and Hummel HE, Host plant selection of the Colorado potato beetle as influenced by damage induced volatiles of the potato plant. *Naturwissenschaften* 84:212–217 (1997).
- 44 Dickens JC, Orientation of Colorado potato beetle to natural and synthetic blends of volatiles emitted by potato plants. *Agric Forest Entomol* 2:167–172 (2000).
- 45 Dickens JC, Behavioural responses of larvae of Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), to host plant volatile blends attractive to adults. *Agric Forest Entomol* 4:309–314 (2002).
- 46 DeWilde J, Lambers-Suwerkropp HR and Van Tol A, Responses to airflow and airborne plant odour in the Colorado beetle. *Neth J Plant Pathol* 75:53–57 (1969).
- 47 Edwards MA and Seabrook WD, Evidence for an airborne sex pheromone in the Colorado potato beetle, *Leptinotarsa decemlineata*. *Can Entomol* 129:667–672 (1997).
- 48 Boiteau G, Sperm utilization and post-copulatory female-guarding in the Colorado potato beetle. *Leptinotarsa decemlineata*. *Entomol Exp Appl* 47:183–187 (1988).
- 49 Dickens JC, McGovern WL and Wiygul G, Effects of antennectomy and a juvenile hormone analog on pheromone production in the boll weevil, *Anthonomus grandis* Boh (Coleoptera: Curculionidae). *J Entomol Sci* 23:52–58 (1988).
- 50 Martel JW, Alford AR and Dickens JC, Alternative management of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), using a host plant volatile-based attractant. *J Econ Entomol* (in press).
- 51 Arthur AP, Associative learning by *Nemeritis canescens* (Hymenoptera: Ichneumonidae). *Can Entomol* 103:1137–1141 (1971).
- 52 Cornell H and Pimentel D, Switching in the parasitoid *Nasonia vitripennis* and its effects on host competition. *Ecology* 59:297–308 (1978).
- 53 Drost YC, Lewis WJ, Zanen PO and Keller MA, Beneficial arthropod behavior mediated by airborne semiochemicals: flight behavior and influence of preflight handling of *Microplitis croceipes* (Cresson). *J Chem Ecol* 12:1247–1262 (1986).
- 54 Drost YC, Lewis WJ and Tumlinson JH, Beneficial arthropod behavior mediated by airborne semiochemicals. *J Chem Ecol* 14:1607–1616 (1988).
- 55 Eller FJ, Tumlinson JH and Lewis WJ, Beneficial arthropod behavior mediated by semiochemicals. II. Olfactometric studies of host-location by the parasitoid *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae). *J Chem Ecol* 14:421–430 (1988).
- 56 Herard F, Keller MA, Lewis WJ and Tumlinson JH, Beneficial arthropod behavior mediated by airborne semiochemicals. IV. Influence of host diet on host-oriented flight chamber responses of *Microplitis demolitor* (Wilkinson). *J Chem Ecol* 14:1597–1606 (1988).
- 57 Strand MR and Vinson SB, Behavioral responses of the parasitoid *Cardiophiles nigriceps* to a kairomone. *Entomol Exp Appl* 31:308–315 (1982).
- 58 Vet LEM and Van Opzeeland K, The influence of conditioning on olfactory microhabitat and host location in *Asobara tabida* (Nees) and *A. rufescens* (Foerster) (Braconidae: Alysiinae) larval parasitoids of Drosophilidae. *Oecologia* 63:171–179 (1984).
- 59 Vet LEM, Responses to kairomones by some alysiinae and eucoilid parasitoid species (Hymenoptera). *Neth J Zool* 35:486–496 (1985).
- 60 Wesloh RM (Ed) *Host location by parasitoids, in Semiochemicals: their role in pest control*, John Wiley and Sons, New York, USA, (1981).
- 61 Lewis WJ and Tumlinson JH, Host detection by chemically mediated associative learning in a parasitic wasp. *Nature (London)* 331:257–259 (1988).
- 62 Turlings TCJ, Tumlinson JH and Lewis WJ, Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science (Washington)* 250:1251–1253 (1990).
- 63 Dicke M, Van Keed TM, Posthumus MA, Ben Dom N, Van Dokhoven H and De Groot AE, Isolation and identification of volatile kairomone that affects acarine predator-prey interactions. Involvement of host plant in its production. *J Chem Ecol* 20:411–425 (1990).
- 64 Takabayashi J, Dicke M, Takahashi S, Posthumus MA and Van Beek TA, Leaf age affects composition of herbivore-induced synomones and attraction of predatory mites. *J Chem Ecol* 20:373–386 (1994).
- 65 Turlings TCJ, McCall PJ, Alborn HT and Tumlinson JH, An elicitor in caterpillar oral secretions that induces corn seedlings to emit chemical signals attractive to parasitic wasps. *J Chem Ecol* 19:411–425 (1993).
- 66 De Moraes CM, Lewis WJ, Paré PW, Alborn HT and Tumlinson JH, Herbivore-infested plants selectively attract parasitoids. *Nature (London)* 393:570–573 (1998).

- 67 Paré PW and Tumlinson JH, Induced synthesis of plant volatiles. *Nature (London)* **385**:30–31 (1997).
- 68 Loughrin JH, Manukian A, Heath RR and Tumlinson JH, Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plants. *Proc Natl Acad Sci USA* **91**:11 836–11 840 (1994).
- 69 Paré PW and Tumlinson JH, *De novo* biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant Physiol* **114**:1161–1167 (1997).
- 70 Paré PW and Tumlinson JH, Plant volatiles as a defense against insect herbivores. *Plant Physiol* **121**:325–331 (1999).
- 71 Turlings TCJ and Tumlinson JH, Systemic release of chemical signals by herbivore-injured corn. *Proc Natl Acad Sci USA* **89**:8399–8402 (1992).
- 72 Rose USR, Manukian A, Heath RR and Tumlinson JH, Volatile semiochemicals released from undamaged cotton leaves: a systemic response of living plants to caterpillar damage. *Plant Physiol* **111**:487–495 (1996).
- 73 Paré PW and Tumlinson JH, Cotton volatiles synthesized and released distal to the site of insect damage. *Phytochemistry* **47**:749–757 (1997).
- 74 Mori N, Tumlinson JH and Teal PEA, Enzymatic decomposition of elicitors of plant volatiles in *Heliothis virescens* and *Helicoverpa zea*. *J Insect Physiol* **47**:749–757 (2001).
- 75 Alborn HT, Jones TH, Stenhagen GS and Tumlinson JH, Identification and synthesis of volicitin and related components from beet armyworm oral secretions. *J Chem Ecol* **26**:203–220 (2000).
- 76 De Moraes CM, Mescher MC and Tumlinson JH, Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature (London)* **410**:577–580 (2001).
- 77 Mattiacci L, Dicke M and Posthumus MA, β -Glucosidase: an elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps. *Proc Natl Acad Sci USA* **92**:2036–2040 (1995).
- 78 Alborn HT, Turlings TCJ, Jones TH, Stenhagen G, Loughrin JH and Tumlinson JH, An elicitor of plant volatiles from beet armyworm oral secretion. *Science (Washington)* **276**:945–949 (1997).
- 79 Pohnert G, Jung V, Haukioja E, Lempa K and Boland W, New fatty acid amides from regurgitant of Lepidopteran (Noctuidae, Geometridae) caterpillars. *Tetrahedron* **55**:11 275–11 280 (1999).
- 80 Kessler A and Baldwin IT, Defensive function of herbivore-induced plant volatile emissions in nature. *Science (Washington)* **291**:2141–2144 (2001).
- 81 Halitschke R, Schittko U, Pohnert G, Boland W and Baldwin IT, Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. III. Fatty acid-amino acid conjugates in herbivore oral secretions are necessary and sufficient for herbivore-specific plant responses. *Plant Physiol* **125**:711–717 (2001).
- 82 Paré PW, Alborn HT and Tumlinson JH, Concerted biosynthesis of an insect elicitor of plant volatiles. *Proc Natl Acad Sci USA* **95**:13 971–13 975. (1998).
- 83 Stanley-Samuelson DW, Prostaglandins and related eicosanoids in insects. *Adv Insect Physiol* **24**:115–212 (1994).
- 84 Ogg CL, Howard RW and Stanley-Samuelson DW, Fatty acid composition and incorporation of arachidonic acid into phospholipids of hemocytes from the tobacco hornworm *Manduca sexta*. *Insect Biochem* **21**:809–814. (1991).
- 85 Blomquist GJ, Borgeson CE and Vundla M, Polyunsaturated fatty acids and eicosanoids in insects. *Insect Biochem* **21**:99–106 (1991).
- 86 Li X, Schuler MA and Berenbaum MR, Jasmonate and salicylate induce expression of herbivore cytochrome P450 genes. *Nature (London)* **419**:712–715 (2002).
- 87 Butenandt A, Beckmenn R, Stramm D and Hecker E, Über den Sexual-Lockstoff des Seidenspinners *Bombyx mori*. Reindarstellung und Konstitution. *Z Naturforschg* **14B**:283–284 (1959).